

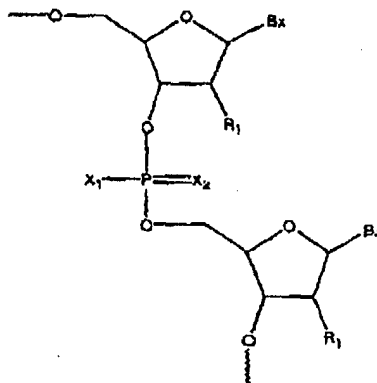
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Application No.: 09/b40,279
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PROCEDURE PURSUANT TO
37 CFR § 1.116

This listing of claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims:

1. (Previously presented) A method of preparing an oligomeric compound having at least one moiety of formula:



wherein:

X₂ is O or S;

X₁ is Pg-O-, Pg-S-, C₁-C₁₀ straight or branched chain alkyl, CH₃(CH₂)_{nn}-O-, R₂R₃N- or a group remaining from coupling a chiral auxiliary;

nn is from 0 to 10;

Pg is CH₃, -CH₂CH₂CN, -C(CH₃)(CH₃)-CCl₃, -CH₂-CCl₃, -CH₂CH=CH₂, CH₂CH₂SiCH₃, 2-yl-ethyl phenylsulfonate, δ-cyanobutenyl, cyano *p*-xylyl, diphenylsilyl-ethyl, 4-nitro-2-yl-ethylbenzene, 2-yl-ethyl-methyl sulfonate, methyl-N-trifluoroacetyl ethyl, acetoxy phenoxy ethyl, or a blocking group;

R₁ is, independently, hydrogen, a blocked hydroxyl group, a sugar substituent group, a nitrogen protecting group, a substituted or unsubstituted C₁-C₁₀ alkyl, a substituted

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or unsubstituted C₂-C₁₀ alkenyl, or a substituted or unsubstituted C₂-C₁₀ alkynyl, wherein said substitution is OR₃, SR₃, NH₃⁺, N(R₃)(R₄), guanidine or acyl where said acyl is an acid amide or an ester;

R₂ is, independently, hydrogen, a C₁-C₁₀ alkyl, a cycloalkyl, an aryl, a nitrogen protecting group, a substituted or unsubstituted C₁-C₁₀ alkyl, a substituted or unsubstituted C₂-C₁₀ alkenyl, or a substituted or unsubstituted C₂-C₁₀ alkynyl, wherein said substitution is OR₃, SR₃, NH₃⁺, N(R₃)(R₄), guanidine or acyl where said acyl is an acid amide or an ester;

or R₁ and R₂ together, are a nitrogen protecting group or are joined in a ring structure;

R₃ is, independently, hydrogen, a C₁-C₁₀ alkyl, a cycloalkyl, an aryl, or a nitrogen protecting group;

R₄ is, independently, N(L₁)L₂, hydrogen, a C₁-C₁₀ alkyl, or a nitrogen protecting group;

or R₃ and R₄, together, are a nitrogen protecting group;

or R₃ and R₄ are joined in a ring structure;

or optionally, R₂ and R₃, together with the nitrogen atom to which they are attached form a cyclic moiety;

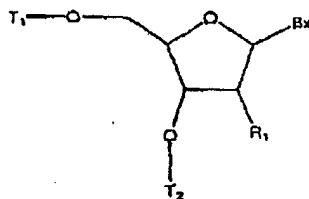
each Bx is, independently, a heterocyclic base moiety; and

comprising the steps of:

(a) providing a 5'-O-protected compound of the formula:

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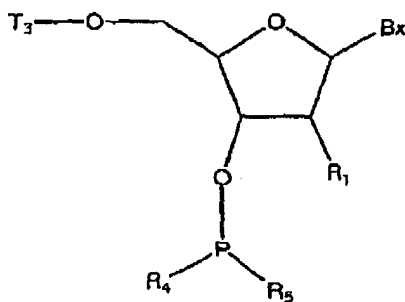
wherein:

T₁ is a hydroxyl protecting group; and

T₂ is a covalent attachment to a support media, a nucleoside bound to a support media, a nucleotide, an oligonucleoside or an oligonucleotide;

(b) treating said 5'-O-protected compound with a deprotecting reagent for a time and under conditions effective to form a 5'-O-deprotected compound;

(c) coupling said 5'-O-deprotected compound with an activated phosphorus composition of the formula:



wherein:

T₃ is a hydroxyl protecting group, a nucleoside, a nucleotide, an oligonucleoside or an oligonucleotide;

each L₁ and L₂ is, independently, C₁₋₆ straight or branched alkyl, or a C₃₋₇ cyclic aliphatic ring system;

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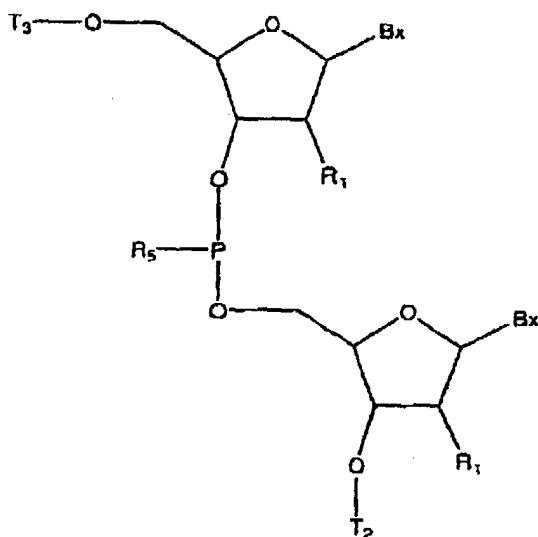
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or L_1 and L_2 are joined together to form a 4- to 13-membered heterocyclic ring system including the nitrogen atom to which L_1 and L_2 are attached; and

R_3 is X_1 ;

or R_4 and R_5 together with the phosphorus atom to which R_4 and R_5 are attached form a chiral auxiliary;

for a time and under conditions effective to form an extended compound having the formula:



(d) treating said extended compound with a mixture comprising an oxidizing reagent and a capping reagent in a single step and for a time and under conditions effective to form said oligomeric compound, and

(e) treating said oligomeric compound with a reagent for a time and under conditions effective to remove said blocking groups thereby forming a deblocked oligomeric compound.

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2. Canceled

2/3. (Currently amended) The method of claim 2 1 wherein said reagent in step (e) is effective to cleave the oligomeric compound from the support media.

3 4. (Currently amended) The method of claim 3 2 wherein said reagent in step (e) is aqueous ammonium hydroxide.

4 5. (Currently amended) The method of claim 2 1 further comprising treating said oligomeric compound with a further reagent for a time and under conditions effective to cleave the oligomeric compound from the support media.

5 6. (Original) The method of claim 1 further comprising treating said oligomeric compound with a deprotecting reagent for a time and under conditions effective to deprotect the T₃ hydroxyl protecting group.

6 7. (Original) The method of claim 1 wherein said mixture comprises from 0.02M to 0.2M oxidizing reagent.

7 8. (Original) The method of claim 7 6 wherein said mixture comprises from 0.1M to 0.2M oxidizing reagent.

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8/9. (Original) The method of claim 1 wherein said oxidizing reagent transfers an oxygen atom.

9/10. (Original) The method of claim 9 wherein said oxidizing reagent is iodine, *m*-chloroperbenzoic acid, iodobenzene diacetate, tetra-*n*-butylammonium periodate, *tert*-butyl hydroperoxide, di-*tert*-butyl hydroperoxide, cumene hydroperoxide, hydrogen peroxide; bis-(*tert*-butyl) peroxide; dinitrogen tetroxide, oxone, molecular oxygen, (1*S*)-(+)-(1*S*)-camphorsulfonyl)oxaziridine or a peracid.

10/11. (Original) The method of claim 10 wherein said oxidizing reagent is iodine, *m*-chloroperbenzoic acid, iodobenzene diacetate, *tert*-butyl hydroperoxide, di-*tert*-butyl hydroperoxide, hydrogen peroxide, oxone, molecular oxygen or a peracid.

11/12. (Original) The method of claim 1 wherein said oxidizing reagent transfers a sulfur atom.

12/13. (Original) The method of claim 12 wherein said oxidizing reagent is 3-amino-1,2,4-dithiazole-5-thione; 3-ethoxy-1,2,4-dithiazoline-5-one; 1,2,4-dithiazolidine-3,5-dione; 3-methyl-1,2,4-dithiazolin-5-one; or dimethylthiuram disulfide.

13/14. (Original) The method of claim 13 wherein said oxidizing reagent is dimethylthiuram disulfide.

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14/15. (Original) The method of claim 1 wherein said capping reagent comprises about one part by volume of either acetic anhydride in acetonitrile or tetrahydrofuran; or chloroacetic anhydride in acetonitrile or tetrahydrofuran; added to about one part by volume of either N-methylimidazole and pyridine in acetonitrile or tetrahydrofuran; or *t*-butylphenoxyacetic anhydride in acetonitrile or tetrahydrofuran.

15/16. (Original) The method of claim 14 wherein said capping reagent comprises about one part by volume of 20% acetic anhydride in acetonitrile mixed with about one part by volume of a solution having 20% N-methylimidazole, 30% pyridine and 50% acetonitrile.

16/17. (Original) The method of claim 1 wherein said mixture comprises dimethylthiuram disulfide, acetic anhydride, acetonitrile, N-methyl imidazole and pyridine.

17/18. (Original) The method of claim 1 wherein said mixture comprises from about 0.05M to 0.2M dimethylthiuram disulfide, about 10% acetic anhydride, about 10% N-methyl imidazole and about 15% pyridine in a suitable solvent.

18/19. (Original) The method of claim 17 wherein said solvent is acetonitrile, toluene, ethyl acetate, tetrahydrofuran, dichloromethane, dichloroethane, dioxane, dimethylacetamide and dimethylformamide.

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19/20. (Original) The method of claim 1 wherein said coupling of the 5'-O-deprotected compound with the activated phosphorus composition is performed in the presence of an activating agent.

20/21. (Original) The method of claim 19 wherein said activating agent is 1-H-tetrazole or 4,5-dicyanomidazole.

21/22. (Original) The method of claim 1 where said cyclic moiety is morpholino or phthalimido.

22/23. (Original) The method of claim 1 wherein each L₁ and L₂ is C₁₋₆ alkyl.

23/24. (Original) The method of claim 22 wherein each L₁ and L₂ is isopropyl.

24/25. (Original) The method of claim 1 wherein L₁ and L₂ are joined together to form a heterocyclic ring system including the nitrogen atom to which said L₁ and L₂ are attached, wherein said ring system optionally includes at least one additional heteroatom selected from O, N and S.

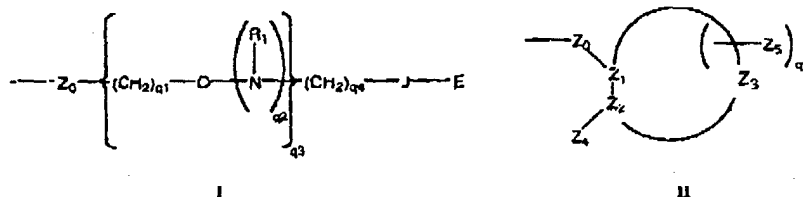
25/26. (Original) The method of claim 25 wherein said heterocyclic ring system is morpholino.

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26/27. (Previously presented) The method of claim 1 wherein each of said sugar substituent groups is, independently, C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₂-C₂₀ alkynyl, C₃-C₂₀ aryl, O-alkyl, O-alkenyl, O-alkynyl, O-aryl, O-aralkyl, O-alkylamino, O-alkylaminoalkyl (O-alkyl-N(H)alkyl), O-alkylaminodialkyl (O-alkyl-N-(alkyl)₂), O-alkylalkoxy (O-alkyl-O-alkyl), O-alkyl-(N-imidazole), thiol, S-alkyl, S-alkenyl, S-alkynyl, NH-alkyl, NH-alkenyl, NH-alkynyl, N-dialkyl, S-aryl, NH-aryl, S-aralkyl, NH-aralkyl, N-phthalimido, halogen keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, N-imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, heterocycle, carbocycle, polyamine, polyamide, polyalkylene glycol, or polyether;

or, alternatively, one or more substituent groups has one of formula I or II:



wherein:

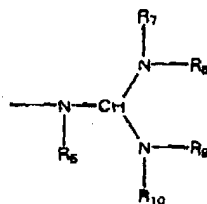
Z₀ is O, S or NH;

J is a single bond, O or C(=O);

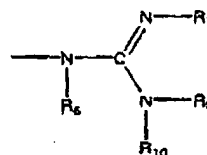
E is C₁-C₁₀ alkyl, N(R₁)(R₂), N(R₁)(R₃), N=C(R₁)(R₂), N=C(R₁)(R₃) or has one of formula III or IV;

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III



IV

each R_6 , R_7 , R_8 , R_9 and R_{10} is, independently, hydrogen, $C(O)R_{11}$, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_2 - C_{10} alkenyl, substituted or unsubstituted C_2 - C_{10} alkynyl, alkylsulfonyl, arylsulfonyl, a chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

or optionally, R_7 and R_8 , together form a phthalimido moiety with the nitrogen atom to which they are attached;

or optionally, R_9 and R_{10} , together form a phthalimido moiety with the nitrogen atom to which they are attached;

each R_{11} is, independently, substituted or unsubstituted C_1 - C_{10} alkyl, trifluoromethyl, cyanoethyloxy, methoxy, ethoxy, *t*-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl;

R_5 is T-L,

T is a bond or a linking moiety;

L is a chemical functional group, a conjugate group or a support media;

each R_1 and R_2 is, independently, H, a nitrogen protecting group, substituted or unsubstituted C_1 - C_{10} alkyl, substituted or unsubstituted C_2 - C_{10} alkenyl, substituted or

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unsubstituted C_2-C_{10} alkynyl, wherein said substitution is OR_3 , SR_3 , NH_3^+ , $N(R_3)(R_4)$,
guanidino or acyl where said acyl is an acid amide or an ester;

or R_1 and R_2 , together, are a nitrogen protecting group or are joined in a ring structure
that optionally includes an additional heteroatom selected from N and O;

or R_1 , T and L, together, are a chemical functional group;

each R_3 and R_4 is, independently, H, C_1-C_{10} alkyl, a nitrogen protecting group, or R_3
and R_4 , together, are a nitrogen protecting group;

or R_3 and R_4 are joined in a ring structure that optionally includes an additional
heteroatom selected from N and O;

Z_4 is OX, SX, or $N(X)_2$;

each X is, independently, H, C_1-C_8 alkyl, C_1-C_8 haloalkyl, $C(=NH)N(H)R_5$,
 $C(=O)N(H)R_5$ or $OC(=O)N(H)R_5$;

R_5 is H or C_1-C_8 alkyl;

Z_1 , Z_2 and Z_3 comprise a ring system having from about 4 to about 7 carbon atoms or
having from about 3 to about 6 carbon atoms and 1 or 2 hetero atoms wherein said hetero
atoms are selected from oxygen, nitrogen and sulfur and wherein said ring system is aliphatic,
unsaturated aliphatic, aromatic, or saturated or unsaturated heterocyclic;

Z_5 is alkyl or haloalkyl having 1 to about 10 carbon atoms, alkenyl having 2 to about
10 carbon atoms, alkynyl having 2 to about 10 carbon atoms, aryl having 6 to about 14
carbon atoms, $N(R_1)(R_2)$ OR₁, halo, SR₁ or CN;

each q_1 is, independently, an integer from 1 to 10,

each q_2 is, independently, 0 or 1;

q_3 is 0 or an integer from 1 to 10;

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q_4 is an integer from 1 to 10;

q_5 is from 0, 1 or 2; and

provided that when q_5 is 0, q_4 is greater than 1.

27 ~~28~~. (Original) The method of claim 1 wherein said X_1 is Pg-O-, Pg-S-, CH_3 -, CH_3 -O-, morpholino or R_2R_3N - where each R_2 and R_3 is, independently, hydrogen or C_1 - C_{10} alkyl.

28 ~~29~~. (Original) The method of claim 1 wherein said Pg is CH_2CH_2CN , diphenylsilylethyl, δ -cyanobutenyl, cyano p-xylyl, methyl-N-trifluoroacetyl ethyl or acetoxy phenoxy ethyl.

29 ~~30~~. (Original) The method of claim 1 wherein said heterocyclic base moiety is adenine, N^6 -benzoyladenine, cytosine, N^4 -benzoylcytosine, 5-methylcytosine, N^6 -benzoyl-5-methylcytosine, thymine, uracil, guanine, N^2 -isobutyrylguanine or 2-aminoadenine.

30 ~~31~~. (Original) The method of claim 1 wherein said support media bound nucleoside, nucleotide, oligonucleoside or oligonucleotide is blocked at reactive sites.

31 ~~32~~. (Original) The method of claim 1 wherein said blocking groups are acid stable.

32 ~~33~~. (Original) The method of claim 1 wherein said blocking groups are base labile.

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³³ 34. (Original) The method of claim 1 wherein said deprotecting reagent is acidic, neutral or basic.

³⁴ 35. (Previously presented) The method of claim ³³ 34 wherein said deprotecting reagent is dichloroacetic acid, trichloroacetic acid, zinc bromide, AlCl_3 , TiCl_4 , $(\text{Et})\text{AlCl}$, $(i\text{-Bu})_2\text{AlCl}$, ceric ammonium nitrate, 1,1,1,3,3,3-hexafluoro-2-propanol or diethyloxomalonate.

³⁵ 36. (Original) The method of claim ³⁴ 35 wherein said deprotecting reagent is 2-5% dichloroacetic acid in dichloromethane or dichloroethane.

³⁶ 37. (Original) The method of claim 1 wherein said deprotecting reagent is a fluoride moiety.

³⁷ 38. (Original) The method of claim ³⁶ 37 wherein said fluoride moiety is BF_3 -etherate.

³⁸ 39. (Original) The method of claim 1 wherein said oligomeric compound comprises from 5 to about 50 nucleosides.

³⁹ 40. (Original) The method of claim 1 wherein said oligomeric compound comprises from 8 to about 30 nucleosides.

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40/41. (Original) The method of claim 1 wherein said oligomeric compound
comprises from 15 to about 25 nucleosides.